Identification of compounds acting as negative allosteric modulators of the LPA 1 receptor

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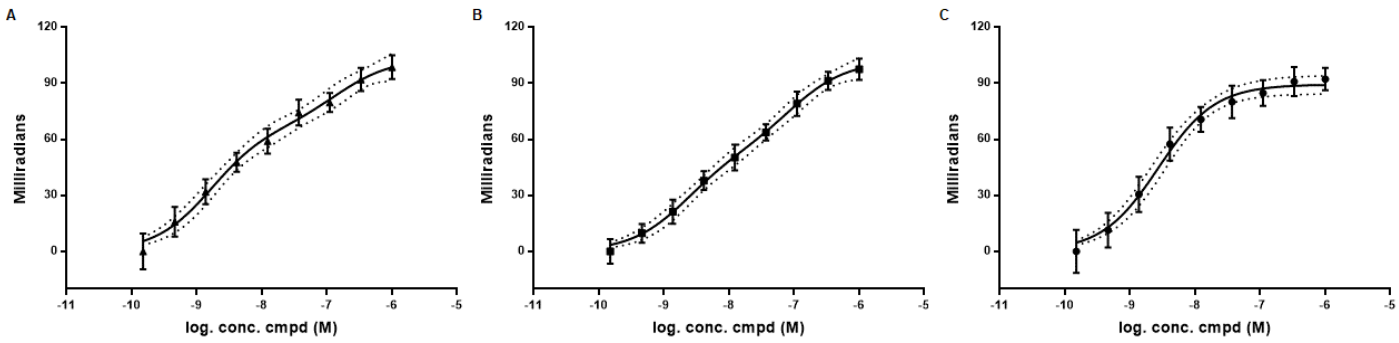

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Fig. 1

The specific binding of TAK-615 (A), SAR-100842 (B) and BMS-896202 (C) to membranes expressing the human LPA_1 receptor. Compounds were incubated with either parental membranes or membranes expressing the LPA_1 receptor. The binding signal was expressed as the phase shift in the interference fringe patterns measured in milliradians. The data from the parental membrane fractions was subtracted from the LPA_1 receptor expressing fractions point by point to obtain the specific binding interaction of the compounds with the LPA_1 receptor. Data points are derived from at least three separate experiments and plotted as means with S.E.M.. Compound affinity was calculated using a comparison fit of a one or two site binding model.

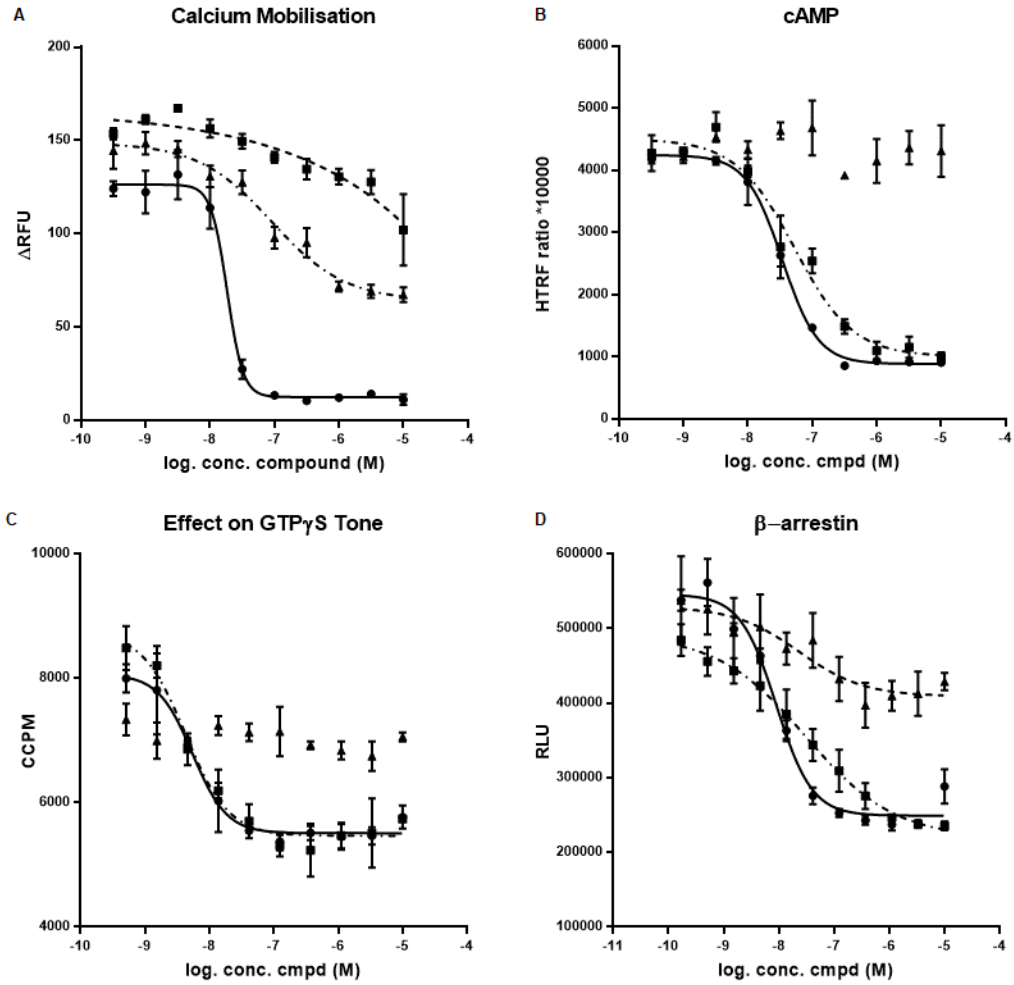

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Fig. 2

Representative graphs to demonstrate the different inhibitory functional profiles of TAK-615 (▲), SAR-100842 (■) & BMS-986202 (●) in a range of LPA driven functional assays using recombinant cell lines expressing the human LPA_1 receptor. In all cases a four-parameter curve fit algorithm was used to define compound potency. (A) Calcium mobilisation assay. Data points are derived from at least quadruplicate wells with S.E.M.. BMS-986202 showed full inhibition, SAR-100842 showed weak inhibition and TAK-615 showed partial inhibition. (B) cAMP accumulation assay. Data points are from duplicate wells. TAK-615 showed no inhibition, SAR-100842 & BMS-986202 showed full inhibition. (C) Millipore GTPγS assay. Data is derived from duplicate wells. SAR-100842 & BMS-986202 showed full inhibition. TAK-615 showed no inhibition. (D) DiscoverX PathHunter β-arrestin2 assay. TAK-615 gave partial inhibition. SAR-100842 & BMS-986202 showed full inhibition.

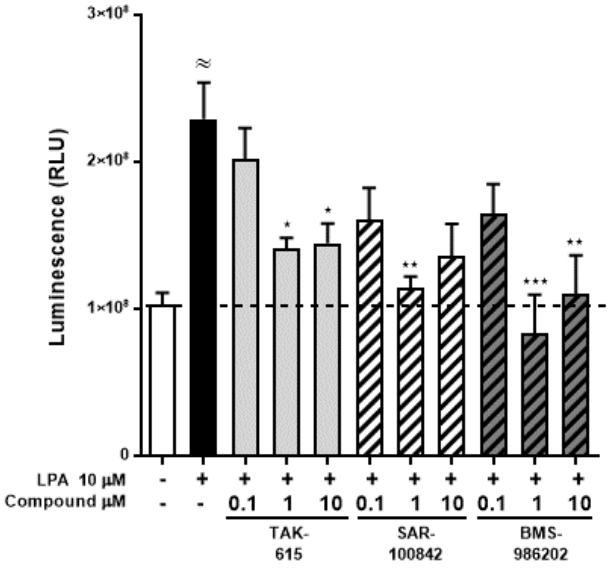

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Fig. 3

Representative data comparing the ability of TAK-615, SAR-100842 and BMS-986202 to inhibit 10 μM LPA stimulated RhoA activation in MeT-5A cells. Data points are derived from quadruplicate wells with S.E.M.. Data was analysed using a one-way ANOVA with a Dunnett's multiple comparisons test. ≈ $P< 0.001$ LPA vs Vehicle. * $P< 0.05$, ** $P< 0.01$, *** $P<0.001$ compound vs LPA.

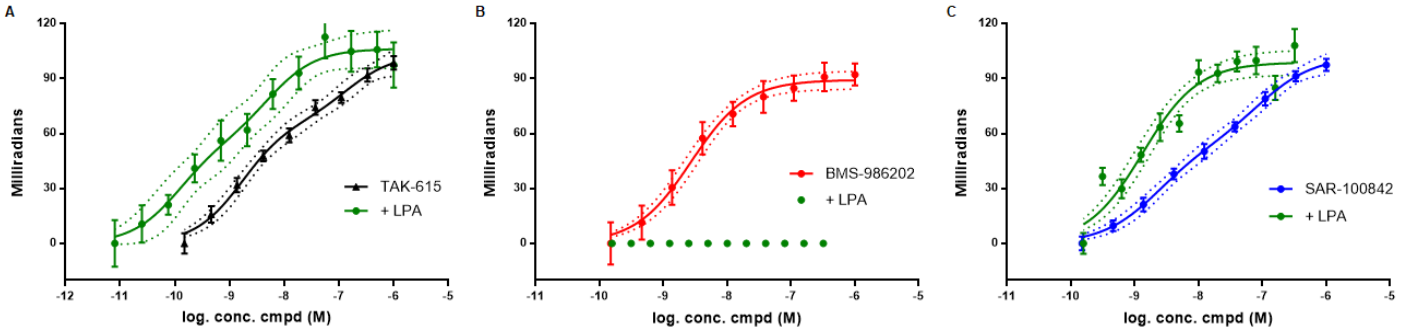

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Fig. 4

LPA increases the apparent affinity of TAK-615, shifts SAR-100842 binding to a single high affinity site but blocks binding of BMS-986202 to the LPA_1 receptor. Parental membranes or membranes expressing the LPA_1 receptor were incubated with a single concentration of LPA (10μM) for 30 mins before addition of TAK-615 (A), SAR-100842 (B) or BMS-986202 (C). The binding signal was expressed as the phase shift in the interference fringe patterns measured in milliradians. The data from the parental membrane fractions was subtracted from the LPA_1 receptor expressing fractions point by point to obtain the specific binding interaction of the compounds with the LPA_1 receptor in the presence or absence of LPA. Data points are derived from at least three separate experiments and plotted as means with S.E.M.. Compound affinity was calculated using a comparison fit of a one or two site binding model.

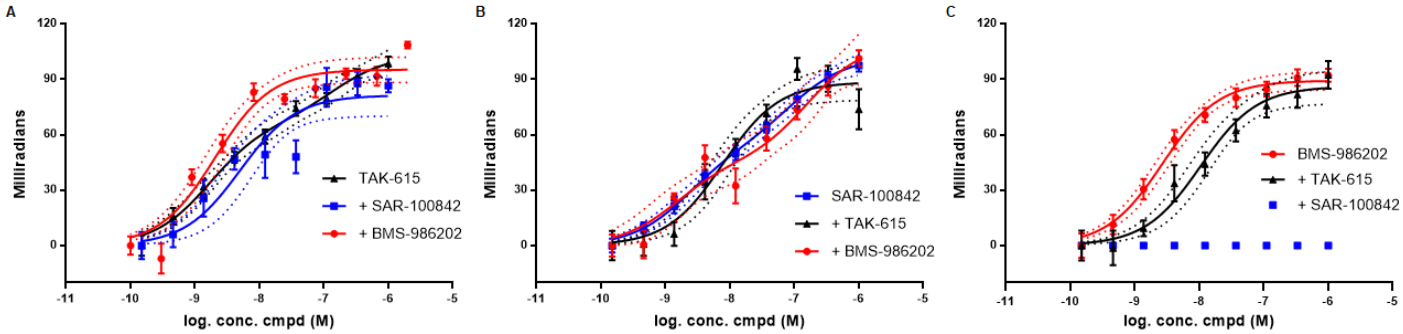

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Fig. 5

Pre-incubating membranes expressing the LPA_1 receptor with SAR-100842 prevents binding of BMS-986202. Parental membranes or membranes expressing the LPA_1 receptor were pre-incubated for 30 mins with 10 μM of a compound before titration with TAK-615 (A), SAR-100842 (B) or BMS-986202 (C). The binding signal was expressed as the phase shift in the interference fringe patterns measured in milliradians. The data from the parental membrane fractions was subtracted from the LPA_1 receptor expressing fractions point by point to obtain the specific binding interaction of the compounds with the LPA_1 receptor in the presence of another compound. Data points are derived from at least three separate experiments and plotted as means with S.E.M.. Compound affinity was calculated using a best fit one or two-site binding model. (A) TAK-615 binding is best described by a one-site model in the presence of SAR-100842 and BMS-986202. (C) TAK-615 decreased the apparent affinity of BMS-986202. SAR-100842 oblates BMS-986202 binding but (B) BMS-986202 has no effect upon SAR-100842 binding. The binding of SAR-100842 in the presence of TAK-615 best fits a one-site model.